Sequence of a *Nicotiana plumbaginifolia* β(1,3)-glucanase gene encoding a vacuolar isoform

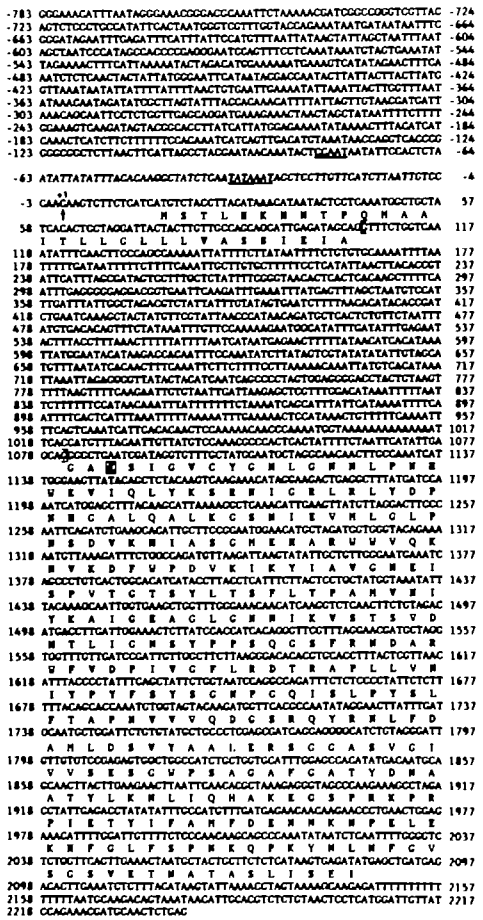
Godelieve Gheysen, Dirk Inzé, Piet Soetaert[1], Marc Van Montagu* and Carmen Castresana
Laboratorium voor Genetica, Rijksuniversiteit Gent, K.L.Ledeganckstraat 35, B-9000 Gent and [1]Plant Genetic Systems N.V., Plateaustraat 22, B-9000 Gent, Belgium

Submitted October 18, 1990 EMBL accession no. X54742

β(1,3)-glucanases are a group of hormonally and developmentally regulated hydrolytic plant enzymes, which are also induced upon pathogen infection. A cDNA and genomic clone of the *Nicotiana plumbaginifolia* β(1,3)-glucanase *gn1* gene have been analyzed previously (1, 2). Here, we report the nucleotide and deduced amino acid sequences of a different *N. plumbaginifolia* β(1,3)-glucanase gene designated *gn2*.

The coding region of *gn2* is contained within two exons and yields a precursor protein of 365 amino acids. In the absence of the corresponding cDNA, the length of the single intron present in *gn2* was determined by primer extension and sequencing of the transcribed mRNA by the dideoxy procedure (3). A synthetic oligonucleotide complementary to nucleotides 16 to 56 downstream from the 5′ end of the second exon was utilized for this experiment. Similarly, primer extension analysis using the same oligonucleotide defined the cap site (arrow in the figure) of the transcript derived from *gn2* at 14 base pairs (bp) upstream from the first ATG of the coding region. The sequences TATAAAT and CCAAT (underlined in the figure) likely corresponding to the *gn2* TATA and CAAT boxes, reside at positions −29 to −35 and −78 to −82 from the cap site, respectively. The N-terminal amino acid sequence of the GN2 protein has previously been determined (4) and the start of the mature GN2 was identified (boxed in figure). The nucleotide sequence data revealed that *gn2* encodes a precursor protein containing a 32-amino acid N-terminal signal peptide for translocation to the endoplasmic reticulum.

Partial amino acid sequence analysis identified two distinct classes of β(1,3)-glucanases in tobacco plants: the extracellular acidic isoforms and the basic isoforms localized primarily in the central vacuole of the cell (5). The *gn2* gene product shows more than 95% identity to the tobacco vacuolar β(1,3)-glucanases, whereas it is only 50−65% homologous to the extracellular isoforms. In addition, *gn2* shows 97.5% identity to the partial cDNA sequence of an intracellular isoform from tobacco (6). These results indicate that GN2 most likely represents a vacuolar β(1,3)-glucanase.



ACKNOWLEDGEMENTS

This work has been supported by a grant from the Services of the Prime Minister (U.I.A.P. 12OC0187). C.C. is indebted to the Commission of European Communities for a postdoctoral fellowship.

REFERENCES

1. De Loose,M., Alliotte,T., Gheysen,G., Genetello,C., Gielen,J., Soetaert,P., Van Montagu,M. and Inzé,D. (1988) *Gene* **70**, 13−23.
2. Castresana,C., de Carvalho,F., Gheysen,G., Habets,M., Inzé,D. and Van Montagu,M. (1990) *The Plant Cell* **2**, in press.
3. Bertholet,C., Van Meir,E., ten Heggeler-Bordier,B. and Wittek,R. (1987) *Cell* **50**, 153−160.
4. Bauw,G., De Loose,M., Inzé,D., Van Montagu,M. and Vandekerckhove,J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4806−4810.
5. Van den Bulcke,M., Bauw,G., Castresana,C., Van Montagu,M. and Vandekerckhove,J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2673−2677.
6. Shinshi,H., Wenzler,H., Neuhaus,J.-M., Felix,G., Hofsteenge,J. and Meins,F.Jr. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5541−5545.

* To whom correspondence should be addressed

Downloaded from http://nar.oxfordjournals.org/ at Ghent University on January 3, 2017